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PRINCIPAL INVESTIGATOR: David J. Waters, Ph.D., DVM

CONTRACTING ORGANIZATION: Purdue Research Foundation
West Lafayette, IN 47907-1021

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13. ABSTRACT (Maximum 200 Words) The goal of this research was to demonstrate the feasibility of the dog model of spontaneous prostate carcinogenesis as a valuable model system to evaluate chemopreventive agents. From April 1, 2002 to March 31, 2003, we have further defined the anticancer effects of the trace mineral selenium on the aging prostate. Our work has generated the first evidence of a non-linear, U-shaped dose : response relationship between selenium status and DNA damage within the prostate. Importantly, the dose : response curve from elderly beagle dogs accurately predicts the relationship between selenium status and prostate cancer risk in men. In our Phase II proposal, we are focusing on interactions between selenium and the antiandrogen, finasteride. To accomplish this, we will conduct a 6 month intervention trial using 60 dogs. At the time of this report, 35 dogs have undergone prostatic biopsy and have been randomly assigned to receive no treatment, selenium alone, finasteride alone, or selenium plus finasteride. Interventions were well tolerated by all dogs. Additional dogs to complete this task will be studied during the project's approved 12 month extension period. Results of biomarker analysis on tissues and body fluids collected pre-treatment, during the experimental period, and at time of euthanasia are pending. Our experience indicates the dog model provides a useful system to study the effects of cancer preventive agents on prostate cells in an appropriate context – <i>in vivo</i> within an aging prostate.						
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INTRODUCTION

Dogs and humans share a vulnerability for the spontaneous development of prostate cancer. Prevention rather than treatment may be the best approach to reduce the morbidity and mortality associated with prostate cancer. Our previous work documented the high prevalence of high-grade prostatic intraepithelial neoplasia in elderly pet dogs and its close association with invasive carcinoma. *In vivo* screening of promising chemopreventive agents using the dog model of spontaneous prostate carcinogenesis represents a novel approach to the prevention of prostate cancer. The goal of this Phase II Idea Development Award is to utilize the dog model to define further the anticancer effects of the trace mineral selenium. The scope of this work includes: (1) continued evaluation of data collected from our Phase I studies on dogs receiving daily supplementation with selenium; and (2) dog experiments testing the extent to which manipulation of the androgen milieu within the prostate (using the 5 α -reductase inhibitor finasteride) significantly influences the response of the aging prostate to selenium supplementation. The long-term objective of this research is to utilize the dog as a pre-clinical model to test innovative ideas in cancer prevention and to further understand the factors that regulate the response of the aging prostate to chemopreventive agents.

BODY

I. Continued Evaluation of Data Collected from Phase I Experiments

What is the Relationship Between Selenium Status and the Level of Genotoxic Stress within the Aging Prostate?

Using the dog model, we have explored the dose : response relationship between selenium status and DNA damage within the prostate. We studied 49 (8.5 – 10.5 year old) sexually intact male, retired breeder dogs that were randomly assigned to either a control group or to receive daily supplementation with selenomethionine or high selenium yeast at 3 or 6 $\mu\text{g}/\text{kg}$ body weight. After 7 months, toenail and prostate tissue specimens were collected immediately after euthanasia and analyzed for total selenium concentration using neutron activation analysis. Dogs from control and selenium treated groups were combined and subdivided into quartiles based on their toenail selenium concentration to evaluate the relationship between toenail selenium level. The extent of DNA damage within the prostate was measured by alkaline Comet assay. There is a non-linear, U-shaped relationship between selenium status and prostatic DNA damage with a relatively narrow range of selenium that optimizes homeostasis within the prostate in terms of DNA damage reduction (**Figure 1**). This U-shaped relationship between micronutrient status and biological response was predicted more than 20 years ago by Mertz [1] (**Figure 2**). According to the Mertz model, a region of optimal nutrient status lies between two suboptimal (low and high) regions and the extreme levels of deficiency and toxicity. Our data provide the first *in vivo* confirmation that Mertz's model is operational for an essential nutrient within the prostate. Importantly, this non-linear U-shaped relationship between selenium status and genotoxic stress within the prostate predicts that not all men will benefit from increasing their selenium status.

Figure 1. Non-linear U-Shaped Relationship Between Prostatic DNA Damage and Selenium Status in 49 Elderly Dogs Physiologically Equivalent to 65-Year Old Men

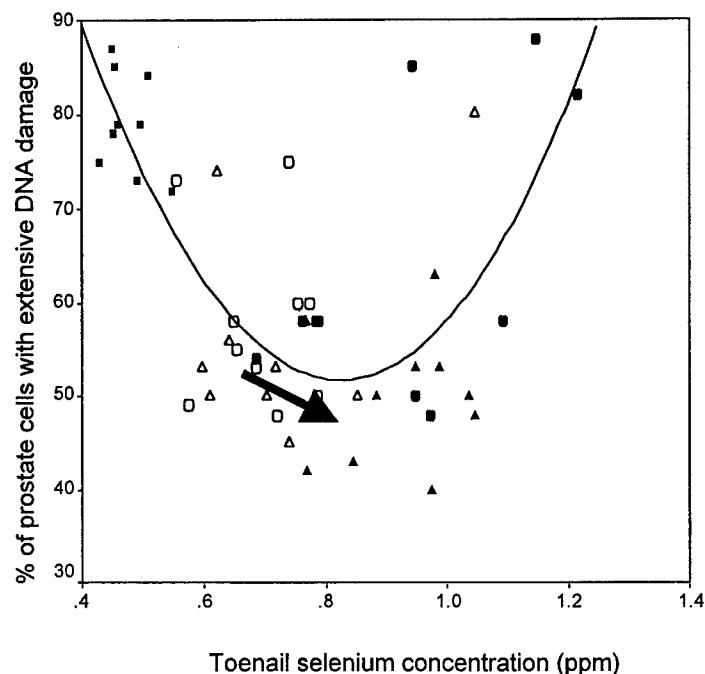
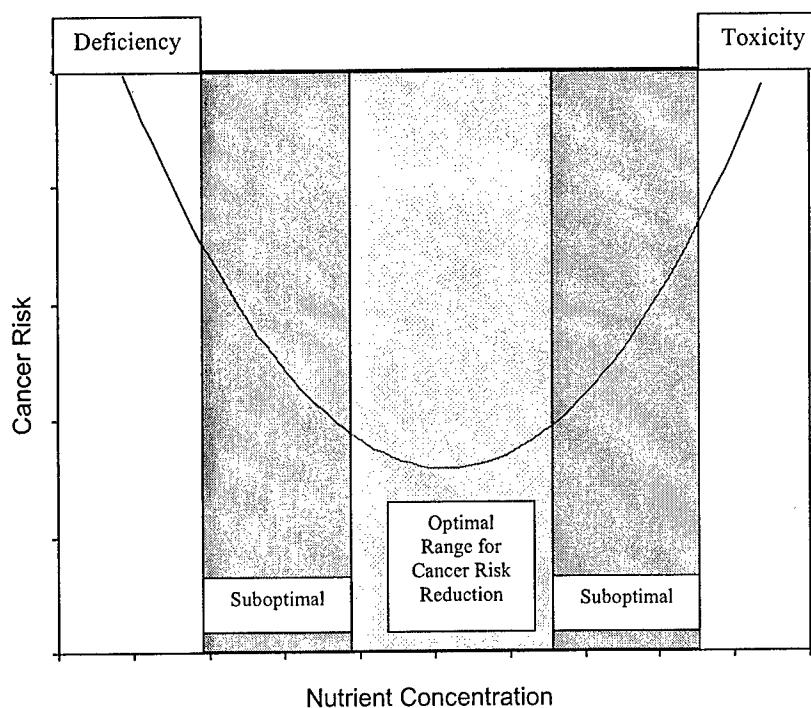


Figure 2. Model Predicting the Biological Response to an Essential Nutrient (adapted from Mertz, 1981)



Does the U-Shaped Relationship Between Toenail Selenium Concentration and Extent of Prostatic DNA Damage in Elderly Beagle Dogs Have Relevance to Selenium Status and Human Prostate Cancer Risk?

Using data from the Health Professionals Follow-Up Study (HPFS), Yoshizawa et al. [2] found a strong inverse association between toenail selenium concentration and risk for advanced prostate cancer. Interestingly, multivariate analysis demonstrated an *apparent threshold effect*, with no additional prostate cancer protective effect at toenail concentrations exceeding 0.82 ppm. In another study, Brooks *et al.* [3] found a similar threshold effect.

We found that toenail concentrations in the lowest and highest quartiles of elderly beagle dogs in our study (mean of 0.50 ppm and 1.03 ppm, respectively) were quite similar to toenail concentrations seen in the HPFS (median of 0.66 ppm in lowest quintile; median of 1.14 ppm in highest quintile). *Fitting the human data from the HPFS to the dog curve produces an intriguing result – the same level of selenium status that minimizes prostatic DNA damage in dogs also minimizes prostate cancer risk in men.* In the HPFS, the highest risk for prostate cancer was in men in the lowest quintile of toenail selenium (median 0.66 ppm) – a value well outside the optimal range predicted by our model. Lowest prostate cancer risk was in men with a median value of 0.82 ppm, which falls within the optimal range of our model. *Thus, movement along our dog curve from low suboptimal to optimal selenium status (bold arrow in Figure 1) was associated with a 65% reduction in human prostate cancer risk.*

In addition, we analyzed data from the Nutritional Cancer Prevention trial of Clark *et al.* [4, 5], converting plasma selenium to an equivalent toenail selenium concentration.¹ Again, the dog curve correctly predicts that men in the lowest tertile of baseline selenium status (<0.71 ppm) would benefit from selenium supplementation. Men in the highest tertile in Clark's study had baseline selenium status (>0.81 ppm) already within the optimum range prior to selenium supplementation; these men did not benefit from selenium supplementation and their post-selenium supplementation selenium status was very high (median, 1.27 ppm).

Taken together, these findings provide strong rationale for using the aging dog prostate to mimic the aging human prostate to further understand the response of prostate cells to selenium. Our results support the hypothesis that toenails are a readily accessible surrogate tissue for monitoring the effects of dietary selenium supplementation on carcinogenic events within the aging prostate. The possibility of a threshold for the prostate cancer protective effects of selenium that can be assayed non-invasively warrants further investigation.

¹ We simultaneously measured toenail and plasma selenium concentration in 12 healthy human volunteers to generate a ratio (6.7 ± 0.7) to convert plasma selenium concentration to predicted toenail values. This technique appears valid because using our conversion, the average plasma selenium concentration in U.S. men (123 ng/ml) is equivalent to a concentration of 0.82 ppm in toenails, which is identical to the median selenium concentration measured in the toenails of men in the HPFS.

II. Progress on Phase II Experiments

TASK 1. To determine if the effect of selenium/antiandrogen on biomarkers of carcinogenesis within the prostate (Months 1-36)

We are in the process of completing our 6 month intervention study in elderly sexually intact male dogs. After prostatic biopsy, 35 dogs have been randomized to 1 of 6 treatment groups: (1) no treatment; (2) selenium supplementation (3 μ g/kg SelenoExcell); (3) selenium plus low dose (0.25 mg/kg/day) finasteride; (4) selenium plus high dose (1mg/kg/day) finasteride; (5) low dose finasteride without selenium; and (6) high dose finasteride without selenium. The 6 month intervention has been completed in 24 dogs. Selenium and finasteride supplementation was well tolerated by all dogs. No technical problems have been encountered. After euthanasia, prostate tissues have been collected for biomarker analysis. Urine, serum, and toenails have also been collected for subsequent measurement of biomarkers. A revised Statement of Work was submitted and approved by Dr. Mishra that addresses some modifications in our laboratory analysis of tissues and body fluids. These include the measurement of total selenium rather than selenium metabolites, and additional assays to assess prostate cell sensitivity to apoptosis.

As an initial step in analyzing our experimental results, we focused on the effects of treatment on prostate volume. For each dog, prostate size in 3 dimensions was measured with calipers prior to treatment and after 6 to 7 months treatment. Prostate weight was calculated using the formula: weight (g) = volume (cm^3) \times 0.602 + 1.16. The anti-trophic effect of finasteride on the prostate was assessed by calculating the percent change in prostate volume over the treatment period. Actual prostate weight recorded at the end of the study was strongly correlated with prostate weight calculated from prostate volume ($r = 0.963$; $p < 0.0001$), validating prostate volume as a robust and reliable index of prostate growth. Dogs in the control group had a median change in prostate volume of +15% over the treatment period. Similarly, dogs receiving supranutritional selenium supplementation had a 16% median increase in prostate volume. In contrast, finasteride-treated dogs had a 42% median reduction in prostate volume after 6 months of treatment ($p < 0.0001$ vs. control group). Finasteride-treated dogs that received supranutritional selenium had a 38% median reduction in prostate volume, which did not differ from dogs treated with finasteride alone ($p=0.52$).

These preliminary data suggest that selenium status does not significantly influence the anti-trophic effects of finasteride on the aging prostate. The dog model enables us to study *in vivo* how differences in selenium status (i.e., nutritionally adequate versus supranutritional) influence prostate cell response to other potential cancer preventive agents. Further analysis of these dogs will determine to what extent the combination of selenium and finasteride affect biomarkers of growth regulation and carcinogenesis within the aging prostate.

TASK 2. To determine the effect of 6 month treatment with selenium/antiandrogen on selenium homeostasis within the prostate and other tissues (Months 1-36)

Serum, toenails, prostate and other tissues are being collected from dogs. Upon completion of sample collection, all samples will be transported to the Morris Laboratory at University of Missouri where total selenium content will be assayed using neutron activation analysis.

Comments: Completion of Task 1 and 2 has been slowed by limited vendor availability of purchased dogs necessary to conduct this research. Recent efforts to increase the availability of dogs have been successful. Therefore, we anticipate that we will be able to complete Tasks 1 & 2 during the 12 month no-cost extension that has been approved by the USAMRMC. To date, our experience strongly supports the feasibility of using the dog model to study how prostate cells respond to potential chemopreventive agents in an appropriate context, i.e. *in vivo* within an aging prostate.

KEY RESEARCH ACCOMPLISHMENTS

- In elderly beagle dogs, there is a non-linear, U-shaped relationship between selenium status and accumulation of DNA damage within the prostate.
- The dose: response curve indicates a relatively narrow optimal range of selenium that maintains prostatic homeostasis, i.e. more selenium is not necessarily better.
- The optimal selenium status predicted by the dog model appears to have implications for human health, because men with the lowest risk of prostate cancer in the Health Professionals Follow-Up study had a median toenail selenium concentration of 0.82 ppm, a value that falls within the optimal range predicted by the dog model.
- The response of the aging prostate to the anti-trophic effects of the 5 α -reductase inhibitor finasteride is not significantly influenced by selenium status.

REPORTABLE OUTCOMES

Manuscripts

Waters DJ, Shen S, Cooley DM, Bostwick DG, Qian J, Combs GF Jr, Glickman LT, Oteham C, Schlittler DL, Morris JS. Effects of dietary selenium supplementation on DNA damage and apoptosis in canine prostate. J Natl Cancer Inst 2003; 95:237-41.

Published Scientific Abstracts

Waters DJ, Shen S, Cooley DM, Bostwick DG, Qian J, Glickman LT, Morris JS. Relationship between toenail concentration of selenium and the level of genotoxic stress within the aging prostate. Environmental and Molecular Mutagenesis 2003; 41: 212.

Shen S, Cooley DM, Glickman LT, Morris JS, Oteham C, Schlittler D, Waters DJ. Reduction of DNA Damage Within the Prostate of Elderly Dogs Receiving Supranutritional Selenium is Associated with Increased Apoptosis of Epithelial Cells. Environmental and Molecular Mutagenesis 2002; 39: 58.

Press Releases

Food for Thought : Selenium's Value to Prostate Health by Janet Raloff. Science News Online, Week of May 3, 2003; Vol. 163, No. 18.

Selenium Lowers Prostatic DNA Damage in Canine Model by John Schieszer. Urology Times, May 2003, Vol. 31, No. 5, p. 4.

Selenium May Fight Prostate Damage by Jennifer Warner. WebMD Medical News, February 4, 2003.

Poster and Oral Presentations

Invited Lectures at National and International Meetings

Dog Models in the Study of Prostatic Disease, American Urologic Association Scientific Retreat, Houston, TX, August 2003.

Relationship Between Toenail Concentration of Selenium and the Level of Genotoxic Stress within the Aging Prostate, Environmental Mutagen Society Annual Meeting Miami Beach, FL, May 2003.

Posters presented at National and International Meetings

Toenail Selenium Concentration is Inversely Associated with the Extent of Prostatic DNA Damage in the Dog Model of Spontaneous Prostate Carcinogenesis. American Association for Cancer Research Frontiers in Cancer Prevention Research Meeting Boston, MA, October 2002.

Reduction of DNA Damage Within the Prostate of Elderly Dogs Receiving Supranutritional Selenium is Associated with Increased Apoptosis of Epithelial Cells. Environmental Mutagen Society 33rd Annual Meeting, Anchorage, Alaska, May 2002.

Effect of Selenium Supplementation on Apoptosis of Prostatic Epithelial Cells in Elderly Dogs. American Association for Cancer Research New Discoveries in Prostate Cancer Biology and Treatment Meeting, Naples, FL, December 2001.

CONCLUSIONS

During the next 12 years, the National Cancer Institute sponsored SELECT trial will study more than 32,000 men to evaluate whether selenium +/- vitamin E will decrease the incidence of human prostate cancer. However, the mechanisms by which selenium modulates key events in the multistep prostate carcinogenesis are unknown. Our work using the dog model yielded the first evidence that daily selenium supplementation can significantly decrease DNA damage within the aging prostate [6]. Furthermore, we showed for the first time that selenium can upregulate apoptosis of prostatic epithelial cells *in vivo* [6]. In our Phase II studies, we are further defining the mechanisms by which selenium supplementation exerts a prostate cancer protective effect. Our work to date takes an important step toward generating important and useful information necessary to develop selenium as a practical means of prostate cancer chemoprevention. Our research addresses a key underexplored area – the further development of an animal model system to study the effects of potential chemopreventive agents on cellular processes that regulate human prostate carcinogenesis. Our most recent findings provide new insight into the complex dose: response relationship between selenium status, genotoxic stress, and carcinogenesis within the aging prostate. Our experience indicates that the response of the human prostate to the anticarcinogenic effects of selenium can be correctly predicted using cost effective short-term studies in dogs, the non-human species most prone to prostate cancer development. This provides a novel approach that can be used to estimate the optimal dose of cancer-fighting micronutrients in order to optimize the design of future interventional trials in men to reduce prostate cancer mortality. The recent evaluation of finasteride in a large prostate cancer prevention trial in 18,000 men has sparked intense interest in the potential anticancer effects of antiandrogens. Completion of our Phase II experiments will provide valuable insights into the consequences of manipulating selenium and androgen status on biomarkers of prostatic carcinogenesis.

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APPENDIX

Manuscripts

Waters DJ, Shen S, Cooley DM, Bostwick DG, Qian J, Combs GF Jr, Glickman LT, Oteham C, Schlittler DL, Morris JS. Effects of dietary selenium supplementation on DNA damage and apoptosis in canine prostate. *J Natl Cancer Inst* 2003; 95:237-41.

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Selenium May Fight Prostate Damage by Jennifer Warner. WebMD Medical News, February 4, 2003.

Scientific Abstracts

Dog Models in the Study of Prostatic Disease, American Urologic Association Scientific Retreat, Houston, TX, August 2003.

Relationship Between Toenail Concentration of Selenium and the Level of Genotoxic Stress within the Aging Prostate, Environmental Mutagen Society Annual Meeting Miami Beach, FL, May 2003.

Reduction of DNA Damage Within the Prostate of Elderly Dogs Receiving Supranutritional Selenium is Associated with Increased Apoptosis of Epithelial Cells. Environmental Mutagen Society 33rd Annual Meeting, Anchorage, Alaska, May 2002.

BRIEF COMMUNICATION

Effects of Dietary Selenium Supplementation on DNA Damage and Apoptosis in Canine Prostate

David J. Waters, Shuren Shen,
Dawn M. Cooley, David G.
Bostwick, Junqi Qian, Gerald F.
Combs, Jr., Lawrence T. Glickman,
Carol Oteham, Deborah Schlittler,
J. Steven Morris

The trace mineral selenium inhibits cancer development in a variety of experimental animal models. We used an *in vivo* canine model to evaluate the effects of dietary selenium supplementation on DNA damage in prostate tissue and on apoptosis in prostate epithelial cells. Sexually intact elderly male beagle dogs were randomly assigned to receive an unsupplemented diet (control group) or diets that were supplemented with selenium (treatment group), either as selenomethionine or as high-selenium yeast at 3 µg/kg or 6 µg/kg body weight per day for 7 months. The extent of DNA damage in prostate cells and in peripheral blood lymphocytes, as determined by the alkaline comet assay, was lower among the selenium-supplemented dogs than among the control dogs (prostate $P < .001$; peripheral blood lymphocytes $P = .003$; analysis of variance) but was not associated with the activity of the antioxidant enzyme glutathione peroxidase in plasma. The median number of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling-positive (i.e., apoptotic) prostate epithelial cells was 3.7 (interquartile range = 1.1–7.6) for the selenium-supplemented dogs and 1.7 (interquartile range = 0.2–2.8) for the control dogs ($P = .04$, Mann-Whitney *U* test). These data suggest that dietary selenium supplementation decreases DNA damage and increases epithelial cell apoptosis within the aging canine

prostate. [J Natl Cancer Inst 2003;95: 237–41]

Prostate cancer is the second leading cause of cancer-related mortality among men in the United States (1). Selenium, an essential nutrient required for the activities of a number of metabolically important enzymes, including the antioxidant glutathione peroxidase, inhibits cancer development in a variety of experimental animal models (2–4). In 2001, the National Cancer Institute initiated the Selenium and Vitamin E Prostate Cancer Prevention Trial (SELECT) to evaluate whether daily dietary supplementation with selenium and/or vitamin E decreases the incidence of prostate cancer. However, it is not known what dietary form or dose of selenium might offer the most potent cancer-protective effects.

Selenium-dependent glutathione peroxidase and thioredoxin reductase protect the body from the endogenous products of cellular metabolism that have been implicated in DNA damage, mutagenesis, and carcinogenesis (5–7). A shift in the pro-oxidant–antioxidant balance within the prostate has been proposed as a factor that contributes to prostate carcinogenesis (8–11). We hypothesized that selenium supplementation exerts its anticarcinogenic effect by reducing the naturally occurring genotoxic stress within the aging prostate. Because the influence of aging on prostate cancer development is similar in dogs and humans, the only two species in which prostate cancer occurs spontaneously with appreciable frequency (12,13), we examined the effects of dietary selenium supplementation on DNA damage and apoptosis in elderly beagle dogs that were physiologically equivalent to 62- to 69-year-old men and free of prostate cancer.

Forty-nine elderly (i.e., 8.5- to 10.5-year-old) sexually intact male, retired breeder dogs weighing 9–18 kg were purchased from a local supplier. After 4 weeks of acclimation, the dogs were randomly assigned to the control group ($n = 10$ dogs), which was fed a maintenance diet that contained 0.3 ppm selenium (Science Diet® Canine Maintenance; Hills Pet Nutrition, Inc., Topeka, KS), or to one of the four daily treatment groups, which received either the maintenance diet plus 3 µg/kg/day selenomethionine (Solgar Vitamin and Herb, Leo-

nia, NJ) ($n = 10$ dogs), 6 µg/kg/day selenomethionine ($n = 10$ dogs), 3 µg/kg/day high-selenium yeast (SelenoEx-cell®; Cypress Systems, Fresno, CA) ($n = 10$ dogs), or 6 µg/kg/day high-selenium yeast ($n = 9$ dogs). The daily selenium intake for the dogs in the control group was approximately 6 µg/kg body weight. All dogs had nutritionally adequate selenium status prior to the start of the experiment [mean pretreatment plasma selenium concentration (14) was 275 ng/mL (range = 228–339 ng/mL)]. The dogs were fed their respective diets for 7 months. At the end of that period, peripheral blood lymphocytes were harvested from whole blood (15–17) that was obtained from each dog, and the dogs were then euthanized in accordance with guidelines set forth by the American Veterinary Medical Association Panel on Euthanasia (18). The prostate was collected *in toto* from each dog within 15 minutes after euthanasia. Prostate tissue (50–80 mg) was harvested fresh to prepare prostate cell suspensions for alkaline comet assay. The remaining prostate was fixed in formalin, embedded in paraffin, and sectioned at 4-mm intervals.

The extent of DNA damage in prostate cells and in peripheral blood lymphocytes was measured by single-cell gel electrophoresis (alkaline comet assay) (19). The extent of DNA damage was visually scored in 100 randomly selected cells from each sample using previously described criteria (20,21) (Fig. 1, A). The ApopTag™ peroxidase *in situ* apoptosis detection kit (Intergen, Inc., Purchase, NY) and a modification of the terminal deoxynucleotidyl trans-

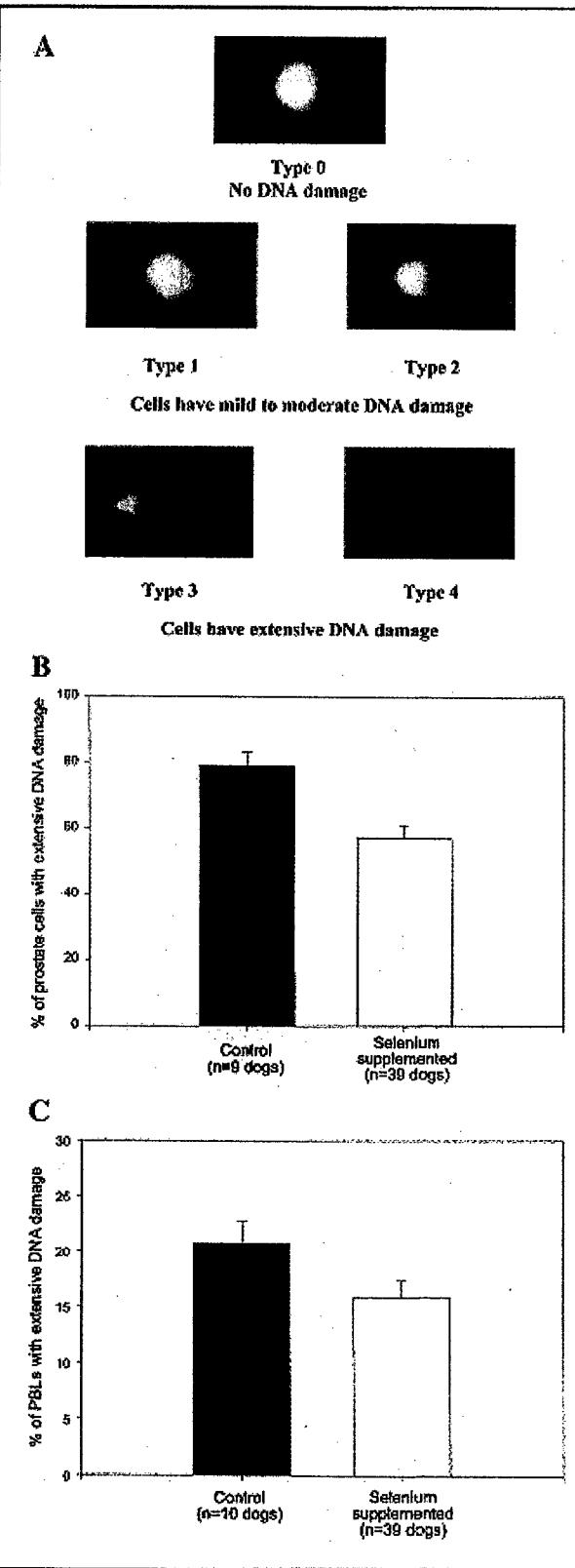
Affiliations of authors: D. J. Waters, S. Shen, D. M. Cooley, Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, and Gerald P. Murphy Cancer Foundation, Seattle, WA; D.G. Bostwick, J. Qian, Bostwick Laboratories, Richmond, VA; G. F. Combs, Jr., Division of Nutritional Sciences, Cornell University, Ithaca, NY; L. T. Glickman (Department of Veterinary Pathobiology), C. Oteham, D. Schlittler (Department of Veterinary Clinical Sciences), Purdue University; J. S. Morris; University of Missouri-Columbia Research Reactor Center, Columbia.

Correspondence to: David J. Waters, D.V.M., Ph.D., Purdue University, School of Veterinary Medicine, 625 Harrison St., West Lafayette, IN 47907-2026 (e-mail: dwaters@gpmcf.org).

See "Notes" following "References."

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Fig. 1. DNA damage in prostate cells and peripheral blood lymphocytes (PBLs) from control dogs and dogs that received daily selenium supplementation. A) Extent of DNA damage in prostate cells and PBLs was measured by single-cell gel electrophoresis (alkaline comet assay) as described by Singh et al. (19). Under the assay conditions used in this experiment, comet tails reflect the electrophoretic migration of DNA fragments that result from strand breaks, alkali-labile sites, crosslinks, or base excision repair sites (19). Extent of DNA damage was scored in 100 randomly selected cells from each sample (50 cells from several different fields from each of two replicate slides) by an examiner who was blinded to treatment group. Each cell was visually scored as previously described (20, 21) according to the following criteria: no damage (type 0), mild to moderate damage (type 1 and type 2), and extensive DNA damage (type 3 and type 4). Extent of DNA damage within prostate cells or PBLs was expressed as the percentage of cells with extensive DNA damage (the total number of cells that displayed type 3 or type 4 DNA damage). B) DNA damage in prostate cells. Within 15 minutes of euthanasia, the prostate was collected from each dog at necropsy, and 50–80 mg of prostate tissue was placed in 1 mL of cold Hanks' balanced salt solution containing 20 mM EDTA and 10% dimethyl sulfoxide (DMSO) (24). One dog in the control group had a tissue sample that was insufficient for further analysis. Tissue was then minced with fine scissors, and 50 µL of the resulting cell suspension was mixed with 1 mL of RPMI-1640 medium containing 10% fetal bovine serum for subsequent electrophoresis. Cytopsin preparations of the cell suspensions indicated that greater than 90% of the cells had an epithelial morphology; the mean percentage of viable cells, as estimated by the trypan blue exclusion assay, was 80%. Bars = mean percentage (and the upper 95% confidence interval) of prostate cells that displayed type 3 or type 4 DNA damage. C) DNA damage in PBLs. PBLs were freshly harvested from whole blood (15–17) that was obtained from each dog after 7 months of treatment and prior to euthanasia. Cytopsin preparations confirmed that more than 90% of the cells in this enriched cell population were lymphocytes; mean percentage of viable cells, as estimated by the trypan blue exclusion assay, was 91%.



ferase-mediated dUTP nick end-labeling (TUNEL) method (22) were used to determine the frequency of apoptosis within sections of dog prostatic tissue. Histopathologic evaluation of formalin-fixed, step-sectioned prostate tissue sections stained with hematoxylin and eosin revealed no foci of carcinoma in any of the dogs. The activity of selenium-dependent glutathione peroxidase in plasma collected immediately prior to euthanasia was assayed by the method of Lawrence and Burk (23) using 0.25 mM H₂O₂ as the acceptor substrate. All aspects of this experimental protocol were approved by the Purdue University Animal Care and Use Committee.

Analysis of variance was used to determine the statistical significance of differences between the control dogs and the selenium-supplemented dogs in the extent of DNA damage in prostate cells or peripheral blood lymphocytes after 7 months on the respective diets. Because no consistent differences in effects were observed with respect to the different forms or doses of selenium the dogs received, in all analyses control dogs were compared with all selenium-supplemented dogs. The median number of apoptotic epithelial cells within prostate tissue sections from control and selenium-supplemented dogs per ×200 microscope field were compared with the use of the Mann-Whitney *U* test. Fisher's exact test was used to compare the percentage of dogs in each treatment group that had more than 30 apoptotic cells per ×200 microscope field. This cutoff point represented a level of apoptosis that exceeded the mean number plus three standard deviations of apoptotic cells in prostate samples from dogs fed the control diet. A *P* value of less than .05 was considered statistically significant, and all tests of statistical significance were two-sided.

After 7 months of treatment, the percentage of prostate epithelial cells and peripheral blood lymphocytes with extensive (i.e., types 3 and 4; Fig. 1) DNA damage was statistically significantly lower in the selenium-supplemented dogs than in the control dogs (mean percentage of prostate cells with extensive DNA damage was 79.1% for the control group and 57.2% for the selenium-treated group [difference = 21.9%, 95% confidence interval [CI] = 13.6% to 30.1%, *P*<.001]; mean percentage of peripheral blood lymphocytes with ex-

tensive DNA damage was 20.7% for the control group and 15.9% for the selenium-treated group [difference = 4.8%, 95% CI = 1.7% to 7.9%, $P = .003$] (Fig. 1, B and C). The mean percentage of prostate cells with extensive DNA damage in dogs in each of the four selenium treatment groups was statistically significantly lower than it was in dogs in the control group (mean percentage of prostate cells with extensive DNA damage was 79.1% for control

dogs and 49.1% for dogs receiving 6 $\mu\text{g}/\text{kg}/\text{day}$ high-selenium yeast [difference = 30.0%, 95% CI = 23.8% to 36.2%, $P < .001$]; 56.9% for dogs receiving 3 $\mu\text{g}/\text{kg}/\text{day}$ high-selenium yeast [difference = 22.2%, 95% CI = 13.5% to 30.9%, $P = .003$]; 63.9% for dogs receiving 6 $\mu\text{g}/\text{kg}/\text{day}$ selenomethionine [difference = 15.2%, 95% CI = 4.0% to 26.4%, $P = .01$]; and 58.1% for dogs receiving 3 $\mu\text{g}/\text{kg}/\text{day}$ selenomethionine [difference = 21.0%, 95% CI = 13.5% to

28.5%, $P < .001$]). After 7 months of treatment, the mean (\pm standard deviation) glutathione peroxidase activity in plasma of control dogs that received a selenium-adequate diet was $25.5 \pm 6.1 \text{ nm/mg protein}$, which was not statistically significantly different from the mean glutathione peroxidase activity in plasma of selenium-treated dogs ($P > .05$).

A very low level of apoptosis was observed within prostate cells from the

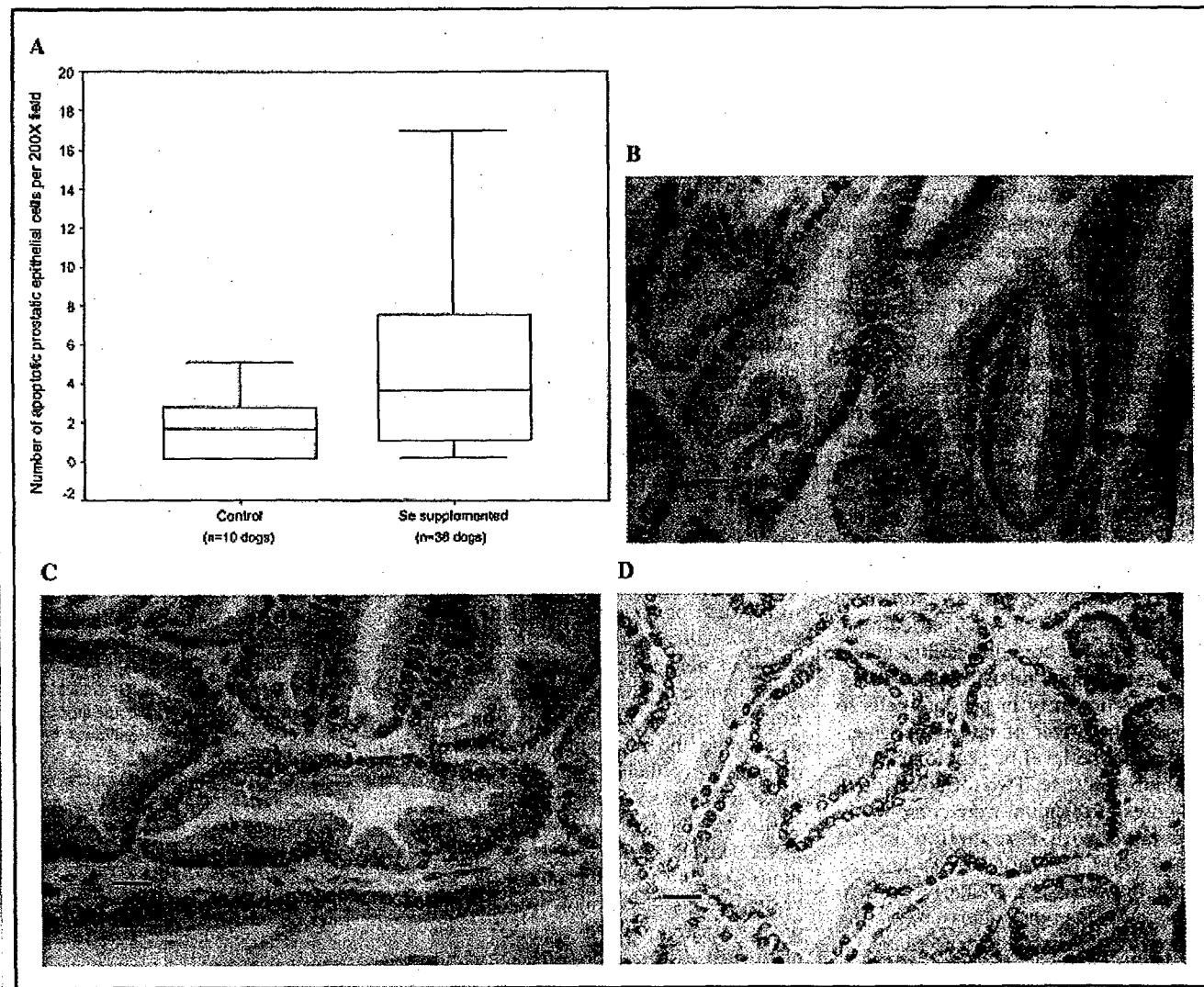


Fig. 2. Prostatic epithelial cell apoptosis in control dogs and dogs receiving daily selenium supplementation. A modified terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) method was used to measure prostatic epithelial cell apoptosis *in situ* in formalin-fixed tissue specimens (22). For each dog, the number of prostate epithelial cells with positive nuclear staining was counted in randomly selected, noncontiguous, $\times 200$ microscopic fields. An average of 23 fields in one tissue section was evaluated for each dog. Immunopositive stromal cells, inflammatory cells, or epithelial cells that were shed into the acinar lumen were not counted. Microscopic fields that contained areas that displayed intense inflammation were not scored. A) Data are displayed in a box and whisker plot (prostate tissue from one selenium-supplemented dog did not react to staining). The center horizontal line indicates the median value

for each group. The length of each box (interquartile range) indicates the range of the central 50% of values, with the box edges placed at the first and third quartiles. Whiskers (the lines extending beyond the box) show the range of observed values that are within 1.5 times the interquartile range. Panels B, C, and D) Representative photomicrographs of TUNEL-stained prostate tissue from a control dog (B) and a selenium-treated dog (C) demonstrate the increased number of epithelial cells with TUNEL-positive nuclear staining (brown) associated with selenium treatment. Panel D shows a region of markedly increased apoptosis ("hot spot") within the prostate of a selenium-treated dog. In each of these $\times 200$ photomicrographs, the scale bar = $50 \mu\text{m}$.

10 control dogs (median number of TUNEL-positive epithelial cells/ $\times 200$ field = 1.7 cells; interquartile range = 0.2–2.8 cells) (Fig. 2, B). By contrast, 38 dogs treated with selenium for 7 months had an approximately twofold increase in the median number of apoptotic cells per field compared with control dogs (median = 3.7 cells; range = 1.1–7.6 cells) ($P = .04$) (Fig. 2, A and C). Foci of increased apoptosis (i.e., apoptotic hot spots), which were defined as those microscopic fields in which there were more than 30 apoptotic cells, were present in prostate tissue sections from 16 (42%) of 38 selenium-supplemented dogs (Fig. 2, D) but in prostate tissue sections from only one (10%) of 10 control dogs ($P = .07$). There were also no statistically significant differences between the two groups of dogs when the cutoff point for apoptotic hotspots was 20, 40, or 50 apoptotic cells per $\times 200$ microscope field ($P = .07$ for each cutoff point).

Our results show that daily supplementation with nontoxic doses of selenium is associated with a decrease in the steady-state level of DNA damage and an increase in epithelial cell apoptosis within the aging canine prostate. Importantly, these effects of selenium supplementation were observed in dogs that had no histologic evidence of prostate cancer and that were of a comparable physiologic age to that of men enrolled in SELECT. We used the alkaline comet assay as a simple, robust method to assess DNA integrity in prostate cells to measure the effect of nutritional intervention on the level of genotoxic stress within the prostate. Two different forms and doses of selenium were consistently associated with a reduction in the steady-state level of DNA damage within the prostate of elderly dogs to levels lower than those measured in the prostate of young adult dogs (data not shown). These biologic responses within the canine prostate were accompanied by statistically significant increases in plasma and toenail selenium concentrations over the treatment period (data not shown). At the end of the study, mean concentration of selenium in toenails collected from selenium-treated dogs was roughly equivalent to the average selenium level found in toenails of men in the Health Professionals Study (data not shown) (25).

The specific mechanism by which selenium supplementation exerts its anticarcinogenic effect on the prostate is unknown (26,27). A reduction in the steady-state level of DNA damage within prostatic epithelial cells could result from a decrease in the rate of DNA damage formation, an increase in the rate or efficiency of DNA damage repair (28), or the preferential elimination of epithelial cells that have the most extensive DNA damage. With regard to the latter possibility, selenium has been shown to induce apoptosis in several *in vitro* models of cancer (27,29–32). Our data support the hypothesis that selenium sensitizes prostatic epithelial cells with extensive DNA damage to apoptosis *in vivo*. Our data also suggest that the effects of selenium on the level of DNA damage are independent of the effects of selenium supplementation on glutathione peroxidase activity. This observation in dogs is consistent with data from a randomized clinical trial of selenium supplementation in humans (14), in which a 63% reduction in prostate cancer incidence was observed in selenium-supplemented men who already had maximal expression of plasma glutathione peroxidase prior to intervention (Combs GF Jr, Clark LC: unpublished data).

In summary, daily supplementation with nontoxic doses of selenomethionine or high-selenium yeast given prior to the development of carcinoma is associated with a reduction in the accumulation of genotoxic damage within the aging canine prostate. Therefore, selenium may benefit the aging prostate by decreasing the accumulation of DNA damage in epithelial cells even before these cells show cytologic changes suggestive of malignancy. We believe that DNA damage and apoptosis are selenium-responsive events that may be important regulatory points in multistep prostatic carcinogenesis. Further study of the process of carcinogenesis within the prostate of animal species vulnerable to spontaneous cancer development may provide important insights into the putative anticancer mechanisms of selenium and identify biomarkers that predict the prostate's response to selenium.

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NOTES

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Week of May 3, 2003; Vol. 163, No. 18

Selenium's Value to Prostate Health

Janet Raloff

Prostate cancer remains the most common malignancy among U.S. men, and internationally it ranks fourth. Though few studies have offered much insight into what triggers this disease, a growing number of researchers have found evidence suggesting that dietary selenium protects men against this cancer.

Indeed, a February 2003 paper in the *International Journal of Cancer* found that among 445 U.S. men, high blood concentrations of selenium appeared to reduce by 30 percent the risk that a man would develop prostate cancer.

Selenium is a constituent of the enzyme glutathione peroxidase, one of the body's more potent antioxidants. Such agents have the ability to quash biologically damaging reactions triggered within the body by any of a host of naturally produced chemicals called oxidants.

Because oxidant damage has been linked with many cancers, some scientists have suspected that any anticancer benefit from selenium probably would trace to its antioxidant contribution.

In fact, however, several new studies suggest that at least one of the nutrient's primary anticancer benefits may be its protection or repair of a suicide switch in genetically damaged cells. It's when the body allows this switch to fail that cancer's runaway growth occurs.

Dogging the problem

David J. Waters of Purdue University and his colleagues were the first to report this discovery in a study of prostate health in elderly beagles. They chose these dogs because, like men, this species spontaneously develops prostate cancer at rates that increase with age.

For 7 months, the scientists supplemented the diets of 38 male dogs—animals physiologically equivalent to 65-year-old men—with either of two dietary supplements: selenomethionine or high-selenium yeast. Another 10 dogs received a similar diet but no extra selenium.



The seafood in this bowl of sushi can be a rich source of selenium. Organ meats are another good source of the mineral.

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At the end of the trial, the researchers sampled blood from each of the animals and then sacrificed the dogs to examine their prostate glands.

Cancers typically trace to DNA damage, and Waters' team found far less of it in the white blood cells and the prostate tissue of dogs treated with selenium than in the untreated group. For instance, 79 percent of the prostate cells examined from untreated dogs had "extensive DNA damage" compared with just 57 percent of such cells from dogs getting supplemental selenium.

However, Waters and his colleagues report in the Feb. 5 *Journal of the National Cancer Institute* that the degree of DNA protection bore no relationship to the activity of glutathione peroxidase in those tissues. "In other words," Waters told *Science News Online*, "[selenium's] beneficial effects cannot be explained by the fact that it was pushing antioxidant enzymes higher."

So how does selenium protect the prostate? It may be by controlling the selective culling of cells with damaged DNA.

Helping cancer cells die

Normally, cells develop, grow old, and then die. Cancer cells, however, don't die naturally. Like Methuselah, they seem immortal and continue to produce endless progeny throughout their long lives.

Cancer cells would pose far less of a problem if the normal suicide switch within them could be reactivated. Such programmed cell death is known as apoptosis. Interestingly, Waters' team found roughly twice the level of apoptosis occurring within the prostate tissue of selenium-supplemented dogs as in untreated beagles. In fact, hot spots of apoptosis appeared in 16 of the 38 treated beagles (42 percent) but just one of the 10 dogs from the untreated group.

The elevated apoptosis in the selenium-treated animals could put a break on the development of prostate malignancies. "The idea here," explains Waters, who also holds a research appointment at the Seattle-based Gerald P. Murphy Cancer Foundation, "is that the cells that are most DNA damaged—and presumably have the highest propensity to turn cancerous—may be selectively purged in the presence of [supplemental] selenium."

The supplementation that conferred this protection was anything but massive. Half the dogs receiving each supplement got a low dose—just 50 percent more than typically occurs in a dog-chow diet and the rest got double the normal dietary selenium supply.

"These are really nontoxic doses," Waters emphasizes. In fact, the lower supplemental dose was roughly equivalent to 200 micrograms per day in men. That's the same amount being administered to some people taking part in a massive, 12-year National Cancer Institute (NCI) nutrition trial. What's more, the forms of selenium tested in the dogs are identical to the forms given to men in earlier trials. In fact, the NCI trial is using selenium methionine.

In the dog trial, the two forms of selenium appeared equally protective, and the low doses were just as good as the high doses.

Why did the Purdue researchers test agents that already have shown their value in people? "Because we want to understand the mechanisms," Waters says, which may point to better doses, the chemical forms that perform best, the ideal timing for supplementation, and whether there will be deleterious interactions between the supplements and drugs or other nutrients in the diet.

Even broccoli may help

John W. Finley and his colleagues at the Agricultural Research Service's Human Nutrition Research Center in Grand Forks, N.D., have been probing a more natural selenium supplement. They aim to deliver anticancer benefits from selenium by enriching the nutrient's concentrations in broccoli (SN: 4/21/01, p. 248; Available to subscribers at <http://www.sciencenews.org/20010421/note12.asp>).

At the Experimental Biology 2003 meeting in San Diego last week, Finley's group reported data from mice that spontaneously develop precancerous tissue in their digestive tract. Animals downing high concentrations of the novel broccoli developed several anticancer changes—among them, the activation of apoptosis-promoting genes.

In another paper at the same meeting, Aimee L. Taylor and her colleagues at Brigham Young University in Provo, Utah, provided data from test-tube studies of prostate cancer cells treated with high concentrations of selenium. Here, too, the nutrient inhibited a series of genes that can turn off the molecular suicide switch in cancer cells.

Looking for other natural sources of this trace mineral? Try seafood, organ meats such as kidney and liver, and to a lesser extent, other meats. Though some grains can be rich stores of selenium, whether they do depends on the mineral status of the soil in which they're grown.

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David J. Waters
School of Veterinary Medicine
Purdue University
625 Harrison Street
West Lafayette, IN 47907-2026

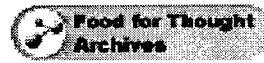
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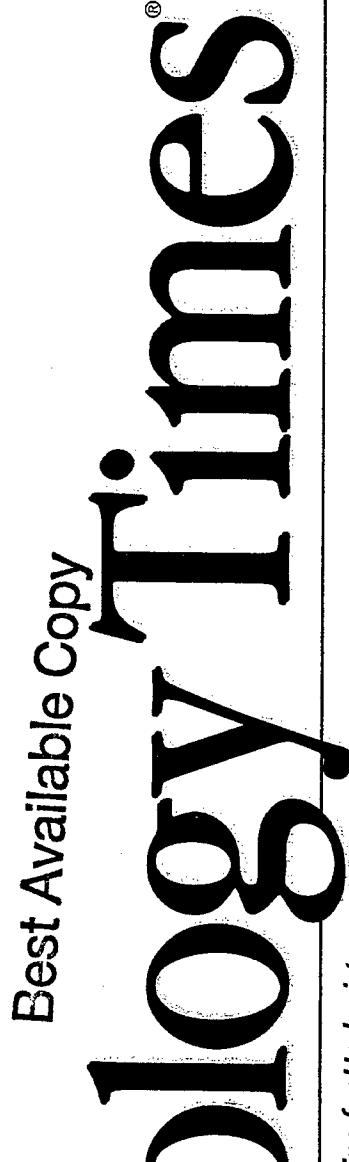
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Lifestyle changes may prevent, reverse PCa

Study recommends changes in diet, even if patients undergo conventional treatment

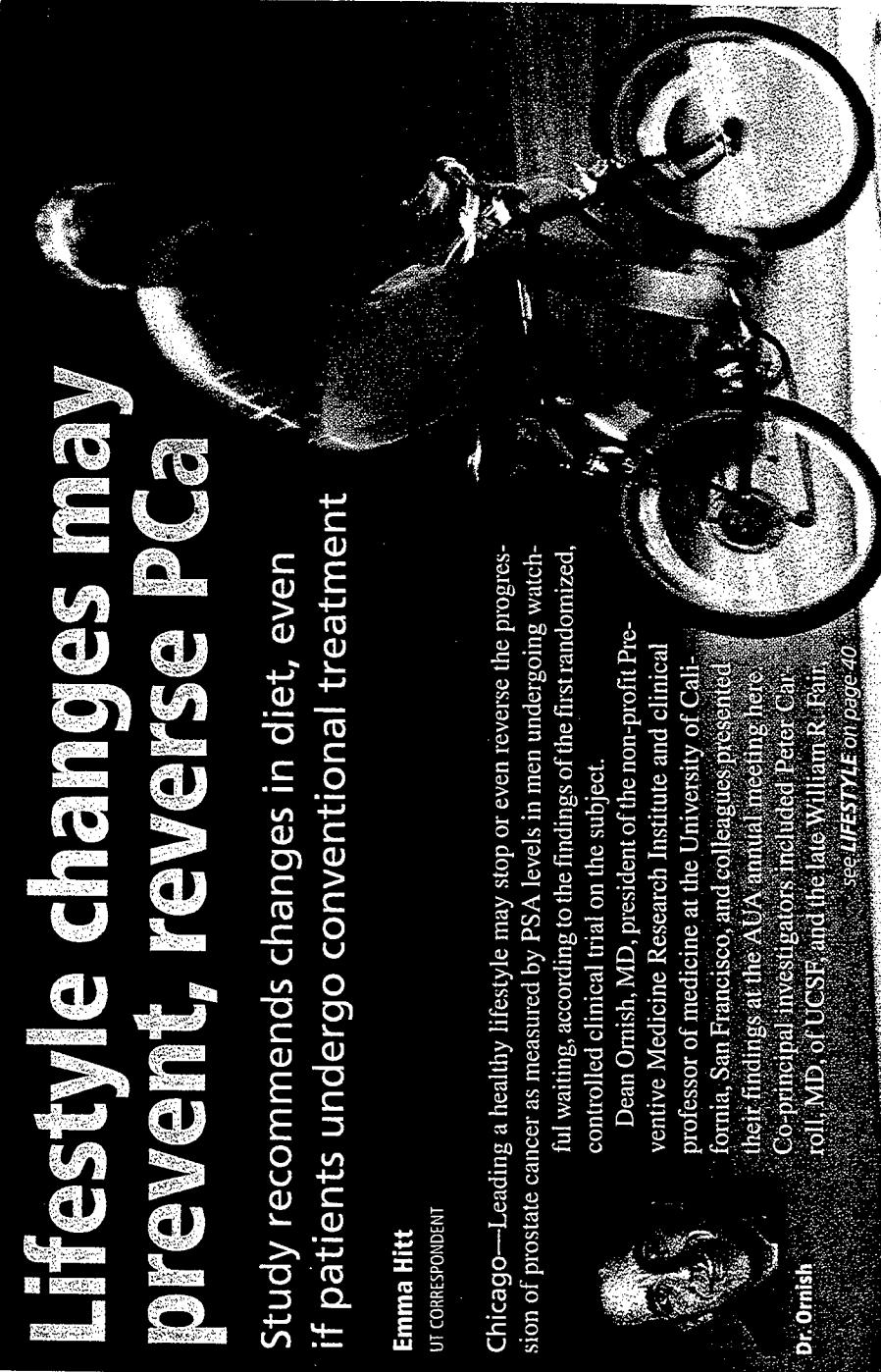
Emma Hitt
UT CORRESPONDENT

Chicago—Leading a healthy lifestyle may stop or even reverse the progression of prostate cancer as measured by PSA levels in men undergoing watchful waiting, according to the findings of the first randomized, controlled clinical trial on the subject.

Dean Ornish, MD, president of the non-profit Preventive Medicine Research Institute and clinical professor of medicine at the University of California, San Francisco, and colleagues presented their findings at the AUA annual meeting here. Co-principal investigators included Peter Carroll, MD, of UCSF and the late William R. Fair,

Dr. Ornish

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Botulinum toxin shown to improve detrusor overactivity in kids, studies show

In a retrospective, multicenter study from Europe, botulinum-A toxin (Botox) injected into the detrusor muscle was shown to allow safe management of detrusor hyperreflexia and neurogenic reflex incontinence.

André Reitz, MD, a resident in neuro-urology at Balgrist University Hospital, Zurich, Switzerland, presented the experience of 200 patients treated in 10 centers for neurourology in Europe. All patients were treated for neurogenic incontinence due to detrusor hyperreflexia. All patients also had resistance or severe side effects to high doses of anticholinergic agents.

Patients were evaluated after botulinum-A injection with clinical examination, urine analysis, and complete urodynamics, said Dr. Reitz. Each patient received botulinum-A injections (total of 300 units diluted in 30 mL saline, 10 units/mL, at 30 sites) into their detrusor muscle, sparing the trigonum. No injection-related or toxin-related side effects were noted.

"We did the injections with local anesthesia, and the procedure lasts about 20 minutes," Dr. Reitz said. "The number of injections until now is experience-based. This is a matter of further research to reduce

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Florida urologist selected to rebuild Iraqi health care

Iraqi exile tapped as interim minister of health

Tim Snider

MANAGING EDITOR

Washington—A Florida urologist is slated to become the highest-ranked health official in Iraq—albeit temporarily.

Said Dr. Hakky, MD, is one of about 60 Iraqi-Americans who have been selected as members of the Iraqi Reconstruction and Development Council, a group working under the Pentagon's Office of Reconstruction and Humanitarian Assistance.

They will be installed as the first authorities of the new Iraqi regime until such time as the country's infrastructure (health, judicial, and social services) is

see **IRAQ** on page 50



Also increases prostate cancer apoptosis

Selenium lowers prostatic DNA damage in canine model

John Schieszer

UT CORRESPONDENT

a growing body of scientific evidence that selenium supplementation may be useful in preventing prostate cancer in men. The data may also heighten anticipation of the findings of a large, multinational study examining the potential preventive effect of selenium and vitamin E against prostate cancer.

"Our work is the first *in vivo* demonstration that selenium can up-regulate cell death within the prostate," said David

Seattle—Selenium, when given as a dietary supplement, decreases DNA damage and increases epithelial cell apoptosis in the prostates of elderly dogs, report researchers from Purdue University and the Gerald P. Murphy Cancer Foundation. While their findings involve animals, the results add to

the growing body of scientific evidence that selenium supplementation may be useful in preventing prostate cancer in men. The data may also heighten anticipation of the findings of a large, multinational study examining the potential preventive effect of selenium and vitamin E against prostate cancer.

"Our work is the first *in vivo* demonstration that selenium can up-regulate cell death within the prostate," said David

Dietary selenium's effect on prostate DNA in dogs

	% PCA cells with DNA damage	plasma glutathione peroxidase activity (nm/min/mg)
Control group	79.1 ± 5.3	24.55 ± 6.56
Selenium-3 µg/kg/day	58.1 ± 9.4 ($p < .0001$)	21.74 ± 4.64
Selenium-6 µg/kg/day	63.9 ± 15 ($p = .01$)	24.21 ± 4.13

SOURCE: DAVID WATERS, DVM, PhD



The Power to Prevent...

UROCIT-K POTASSIUM CITRATE

WAX MATRIX TABLETS 5 & 10 meq

BRIEF SUMMARY, CONSULT THE PACKAGE INSERT FOR FULL PRESCRIBING INFORMATION

INDICATIONS AND USAGE: Potassium citrate is indicated for the management of renal tubular acidosis (RTA) with calcium stones, hypocitraturic calcium oxalate nephrolithiasis of any etiology, and uric acid lithiasis without calcium stones.

CONTRAINDICATIONS: Urocit-K is contraindicated in patients with hyperkalemia (or who have conditions predisposing them to hyperkalemia), as a further rise in serum potassium concentration may produce cardiac arrest. Such conditions include: chronic renal failure, uncontrolled diabetes mellitus, acute dehydration, strenuous physical exercise in unconditioned individuals, adrenal insufficiency, extensive tissue breakdown, or the administration of a potassium-sparing agent (such as triamterene, spironolactone or amiloride).

Urocit-K is contraindicated in patients in whom there is cause for arrest or delay in tablet passage through the gastrointestinal tract, such as those suffering from delayed gastric emptying, esophageal compression, intestinal obstruction or stricture or those taking anticholinergic medication. Because of its ulcerogenic potential, Urocit-K should not be given to patients with peptic ulcer disease.

Urocit-K is contraindicated in patients with renal insufficiency (glomerular filtration rate of less than 0.7 ml/kg/min), because of the danger of soft tissue calcification and increased risk for the development of hyperkalemia.

WARNINGS: HYPERKALEMIA: In patients with impaired mechanisms for excreting potassium, Urocit-K administration can produce hyperkalemia and cardiac arrest. Potentially fatal hyperkalemia can develop rapidly and be asymptomatic. The use of Urocit-K in patients with chronic renal failure, or any other condition which impairs potassium excretion such as severe myocardial damage or heart failure, should be avoided.

INTERACTION WITH POTASSIUM-SAVING DIURETICS: Concomitant administration of Urocit-K and a potassium-sparing diuretic (such as triamterene, spironolactone or amiloride) should be avoided, since the simultaneous administration of these agents can produce severe hyperkalemia.

If there is severe vomiting, abdominal pain or gastrointestinal bleeding, Urocit-K should be discontinued immediately and the possibility of bowel perforation or obstruction investigated.

PRECAUTIONS: INFORMATION FOR PATIENTS:

Physicians should consider reminding the patient of the following:

Waters, DVM, PhD, professor of comparative oncology at Purdue's School of Veterinary Medicine, West Lafayette, IN, and director of the Gerald P. Murphy Foundation, Seattle. "We measured DNA damage and apoptosis, and those both appear to be selenium-responsive biomarkers."

Effect in elderly dogs

Dogs are the only non-human species that spontaneously develops prostate cancer. Also, because of their compressed lifespan, 2 years of antioxidant supplements in dogs is the equivalent of 15 to 20 years in humans.

"We wanted to evaluate the effects of selenium on prostate cells in an appropriate context. Aging increases risk for prostate cancer, so we wanted to study the effects of selenium on the aging prostate.

These dogs are physiologically equivalent to 65-year-old men," said Dr. Waters in an interview with *Urology Times*.

In the study, published in the *Journal of the National Cancer Institute* (2003; 95:237-41), *elderly intact male* *canine*

uterus concentrations during the treatment period.

"This is an important finding. Dogs are the closest animal models to humans when it comes to prostate cancer," said Ian Thompson, MD, professor and chief of urology, University of Texas Health Science Center, San Antonio. "In this new study, we have another example of selenium altering an intermediate biomarker that may predict its efficacy in reducing the risk of prostate cancer."

SELECT to provide answers

In 1996, a study of 1,312 men and women found that men who took selenium to prevent nonmelanoma skin cancer received no benefit from selenium in preventing skin cancer. However, the data showed about a 60% reduction in the number of new cases of prostate cancer in those men taking sele-



gastrointestinal tract, such as those suffering from delayed gastric emptying, esophageal compression, intestinal obstruction or stricture or those taking anticholinergic medication. Because of its ulcerogenic potential, Urocit-K should not be given to patients with peptic ulcer disease.

WARNINGS: HYPERKALEMIA: In patients with impaired mechanisms for excreting potassium, Urocit-K administration can produce hyperkalemia and cardiac arrest. Potentially fatal hyperkalemia can develop rapidly and be asymptomatic. The use of Urocit-K in patients with chronic renal failure, or any other condition which impairs potassium excretion such as severe myocardial damage or heart failure, should be avoided.

INTERACTION WITH POTASSIUM-SPARING DIURETICS: Concomitant administration of Urocit-K and a potassium-sparing diuretic (such as triamterene, spironolactone or amiloride) should be avoided, since the simultaneous administration of these agents can produce severe hyperkalemia.

If there is severe vomiting, abdominal pain or gastrointestinal bleeding, Urocit-K should be discontinued immediately and the possibility of bowel perforation or obstruction investigated.

PRECAUTIONS: INFORMATION FOR PATIENTS:

Physicians should consider reminding the patient of the following:

To take each dose without crushing, chewing or sucking the tablet. To take this medicine only as directed. This is especially important if the patient is also taking both diuretics and digitalis preparations.

To check with physician if there is trouble swallowing tablets or if the tablet seems to stick in the throat. To check with the doctor at once if tarry stools or other evidence of gastrointestinal bleeding is noticed.

LABORATORY TESTS: Regular serum potassium determinations are recommended. Careful attention should be paid to acid-base balance, other serum electrolyte levels, the electrocardiogram, and the clinical status of the patient, particularly in the presence of cardiac disease, renal disease or acidosis.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Long-term carcinogenicity studies in animals have not been performed.

PREGNANCY CATEGORY C: Animal reproduction studies have not been conducted with Urocit-K. It is also not known whether Urocit-K can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Urocit-K should be given to a pregnant woman only if clearly needed.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: Some patients may develop minor gastrointestinal complaints during Urocit-K therapy, such as abdominal discomfort, vomiting, diarrhea, loose bowel movements or nausea.

OVERDOSAGE: In overdosage of Urocit-K, treat for hyperkalemia.

DOSAGE AND ADMINISTRATION: Treatment with Urocit-K should be added to a regimen that limits salt intake (avoidance of foods with high salt content and/or added salt at the table) and encourages high fluid intake (urine volume should be at least two liters per day). The objective of treatment with Urocit-K is to provide Urocit-K in sufficient dosage to restore normal urinary citrate (greater than 320 mg/day) and as close to the normal mean of 640 mg/day, as possible), and to increase urinary pH to a level of 6.0 to 7.0.

In patients with severe hypochlorthuria (urinary citrate of less than 150 mg/day), therapy should be initiated at a dosage of 60 meq/day (20 meq three times/day or 15 meq four times/day with meals or within 30 minutes after meals or bedtime snack). In patients with mild-moderate hypochlorthuria (>150 mg/day), Urocit-K should be initiated at a dosage of 30 meq/day (10 meq three times/day with meals). Twenty-four hour urinary citrate and/or urinary pH measurements should be used to determine the adequacy of the initial dosage and to evaluate the effectiveness of any dosage change. In addition, urinary citrate and/or pH should be measured every four months. Doses of Urocit-K greater than 100 meq/day have not been studied and should be avoided.

HOW SUPPLIED: Urocit-K is available for oral administration in tablet form in the following sizes: (NDC 0178-0650-01) 5 meq potassium citrate and (NDC 0178-0610-01) 10 meq potassium citrate, packaged in bottles of 100 each.

Rx ONLY

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2 years of antioxidant supplements in dogs is the equivalent of 15 to 20 years in humans.

"We wanted to evaluate the effects of selenium on prostate cells in an appropriate context. Aging increases risk for prostate cancer, so we wanted to study the effects of selenium on the aging prostate. These dogs are physiologically equivalent to 65-year-old men," said Dr. Waters in an interview with *Urology Times*.

In the study, published in the *Journal of the National Cancer Institute* (2003; 95:237-41), sexually intact, elderly male beagle dogs were randomly assigned to receive an unsupplemented diet (control group) or diets that were supplemented daily with selenium (treatment group) for 7 months—either as selenomethionine or as high-selenium yeast. After 7 months, the extent of DNA damage in canine prostate cells and in peripheral blood lymphocytes, as determined by the alkaline comet assay, was lower among the selenium-supplemented dogs than among the controls.

However, Dr. Waters said that the lower extent of DNA damage in the treatment group was not associated with the activity of the antioxidant enzyme glutathione peroxidase in plasma.

"Glutathione peroxidase in these dogs was already maxed out prior to supplementation. Then, by supplementing with selenium, you are changing something that affords the prostate with a DNA damage protective effect, and that is the mechanism we and others are trying to figure out," Dr. Waters said.

The two different forms and doses of selenium were both associated with a reduction in the steady-state level of DNA damage within the prostate of the elderly dogs. Some reductions were to below the levels of those measured in the prostates of young adult dogs, said Dr. Waters.

In addition, these biological responses were accompanied by statistically significant increases in plasma and toenail selenium and other potential cancer preventive agents.**UT**

altering an intermediate biomarker that may predict its efficacy in reducing the risk of prostate cancer."

SELECT to provide answers

In 1996, a study of 1,312 men and women found that men who took selenium to prevent nonmelanoma skin cancer received no benefit from selenium in preventing skin cancer. However, the data showed about a 60% reduction in the number of new cases of prostate cancer in those men taking selenium compared with those who did not take the supplement (*JAMA* 1996; 276:1957-63). The findings from this study were major contributing factors to the beginning of the Selenium and Vitamin E Cancer Prevention Trial (SELECT), which is now underway. SELECT is designed to determine if selenium, vitamin E, or both of these dietary supplements can prevent prostate cancer in men. The study, which is coordinated by the Southwest Oncology Group, includes more than 32,000 men in the United States, Puerto Rico, and Canada and is being conducted at more than 400 sites internationally.

In SELECT, men on selenium supplementation will receive 1-selenomethionine, 200 µg/day. The dose would have to be increased to more than 2,400 µg/day of selenium to be considered "too much," Dr. Thompson said, adding that he does not recommend even 200 µg/day for any of his patients at this time.

"When SELECT is done we will know the answer," Dr. Thompson said. "While the evidence regarding selenium suggests that it is safe and may have an effect, it will take the SELECT trial to determine the final answers to these questions."

Follow-up studies by Dr. Waters' group are now underway looking at different forms of selenium, varying doses of selenium, and the interactions between selenium and other potential cancer preventive agents.**UT**

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Selenium May Fight Prostate Damage

Supplementation May Reduce Prostate Cancer Risk

By Jennifer Warner

WebMD Medical News

Reviewed By Brunilda

Nazario, MD

on Tuesday, February 04, 2003

Feb. 4, 2003 -- Boosting the daily dose of selenium may help elderly men keep their prostates healthy and reduce their risk of prostate cancer. A new study shows elderly dogs that ate a diet supplemented with selenium had less age-related DNA damage to their prostates, which may reduce the risk of prostate cancer.

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Selenium is an essential nutrient required in small amounts by the human body for a number of vital processes, including some that are thought to reduce the risk of certain types of cancer. The most common dietary sources of selenium are meats, fish, cereal, dairy products, and eggs.

Researchers say that the effect of aging on prostate cancer development is similar in dogs and humans -- the only two species in which prostate cancer occurs spontaneously and with significant frequency. In fact, prostate cancer is the second leading cause of cancer death among men in the U.S.

In the study, researchers selected elderly beagle dogs that were comparable to 62- to 69-year-old men and fed them either a diet that had been supplemented with selenium or a regular diet for seven months and compared the effects of the diets on their prostates.

The results appear in the Feb. 5 issue of the *Journal of the National Cancer Institute*.

Researcher David J. Waters, DVM, PhD, of the school of veterinary medicine at Purdue University, and colleagues found that dogs fed the supplemented diet had a significantly lower percentage of prostate cells with extensive DNA damage than the others. About 80% of the prostate cells in dogs fed a normal diet had extensive DNA damage compared with only about 57% in the selenium-treated dogs.

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In addition, dogs fed the enriched diet also had a twofold increase in the number of prostate cells that had undergone a process that removes damaged cells, called apoptosis, which is associated with a lower risk of cancer.

Researchers say the results show that selenium may help protect cells within the aging prostate from initial DNA damage before the cells develop major problems that might lead to cancer, but more research is needed to see if the same effect holds true in humans.

SOURCES: *Journal of the National Cancer Institute*, Feb. 5, 2003 • WebMD Medical News: "[Mineral Cuts Bladder Cancer in Smokers](#)."

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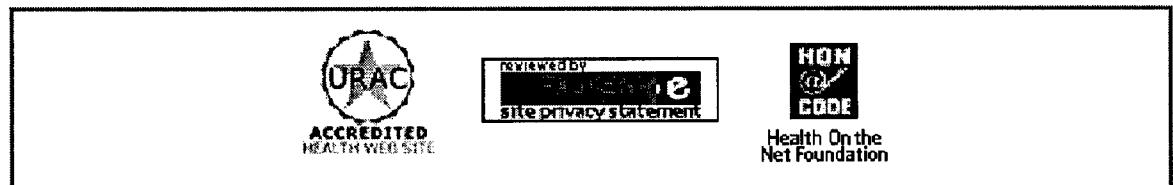


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American Urologic Association Scientific Retreat, Houston, TX, August 2003

Dog Models in the Study of Prostatic Disease

David J. Waters, DVM, PhD

Professor of Comparative Oncology, Purdue University

Director, Gerald P. Murphy Cancer Foundation

An aging-related dysregulation of homeostasis renders the prostates of older dogs and men vulnerable to benign prostatic hyperplasia and prostate cancer. The naturally-occurring carcinomas that arise within the dog prostate mimic their human counterpart with regard to heterogeneity, age at diagnosis, association with high-grade prostatic intraepithelial neoplasia (HGPIN), and propensity for skeletal metastases (1-3). Dogs provide an autochthonous, large animal model for imaging and therapeutic studies. The absence of a standard treatment regimen for pet dogs with prostate carcinoma provides an opportunity to evaluate novel therapeutic strategies against spontaneous prostate cancer. The sporadic incidence of prostate carcinoma within the pet dog population precludes the design and execution of large randomized trials. However, establishing proof of principle of tumor targeting approaches is feasible using a small number of animals. Possible approaches include intralesional (e.g. prostate gland or metastases) or systemic therapies directed against specific targets overexpressed by most human prostate cancers.

Because the incidence of prostate cancer has reached near epidemic proportions, the identification of safe, non-toxic compounds for chemoprevention is a high research priority. In 2001, the National Cancer Institute initiated a 12-year clinical trial (SELECT) that will study more than 32,000 men to evaluate whether daily supplementation with selenium +/- vitamin E decreases the incidence of prostate cancer. However, the mechanisms of how selenium exerts its anticancer effects are unknown. We have used elderly beagle dogs, physiologically equivalent to 65-year old men, in order to study the effects of selenium on prostate epithelial cells in an appropriate context, i.e. *in vivo* within an aging prostate. Using this approach, we have studied the extent to which daily selenium supplementation influences processes (e.g. DNA damage, apoptosis, proliferation) that are fundamentally important in the development and progression of human prostate cancer. Recently, we reported that 7 months of daily selenium supplementation (in the form of high selenium yeast or selenomethionine) significantly reduced the accumulation of DNA damage within the prostate (4). This DNA damage-sparing effect of selenium was accompanied by an increase in apoptosis of epithelial cells within the prostate. We believe that DNA damage and apoptosis are selenium-responsive events that may be important regulatory points in multi-step prostatic carcinogenesis. Additional studies using animal and cellular models to complement ongoing cancer prevention trials in men will provide important insights into the putative anticancer mechanisms of selenium and identify biomarkers that reliably predict the prostate's response to selenium.

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Environmental Mutagen Society Annual Meeting Miami Beach, FL, May 2003

Relationship Between Toenail Concentration of Selenium and the Level of Genotoxic Stress within the Aging Prostate

D.J. Waters^{1,5}, S. Shen^{1,5}, D.M. Cooley^{1,5}, D.G. Bostwick³, J. Qian³, L.T. Glickman², J.S. Morris⁴

Departments of Veterinary Clinical Sciences¹ and Veterinary Pathobiology², Purdue University, West Lafayette, IN, 47907; Bostwick Laboratories³, Richmond, VA 23294; University of Missouri-Columbia Research Reactor Center⁴, Columbia, MO, 65211; Gerald P. Murphy Cancer Foundation⁵, Seattle, WA 98125.

Key Words: chemoprevention, dog, comet assay

ABSTRACT

Analysis of toenails from men in the Health Professionals Follow-up Study showed an inverse association between selenium (Se) status and risk for advanced prostate cancer, with no additional reduction in prostate cancer risk in men with toenail Se concentration exceeding 0.85 ppm. In a previous study, we found that daily supplementation with selenomethionine or high Se yeast significantly reduced DNA damage within the prostate of dogs. The objective of this study was to determine if toenail Se concentration is a surrogate biomarker predictive of the extent of genotoxic stress within the prostate measured by alkaline comet assay. We studied 49 (8.5 – 10.5 year old) male dogs that were randomly assigned to a control group or to receive daily supplementation with Se at 3 or 6 µg/kg body weight. After 7 months, toenail and prostate tissue specimens were analyzed for total Se concentration using neutron activation analysis. Dogs from control and Se treated groups were combined and subdivided into quartiles based on their toenail Se concentration. Dogs with the lowest toenail Se concentration had the highest genotoxic stress within the prostate ($p < 0.0001$). There was a significant inverse correlation between the percentage of cells with extensively damaged DNA and toenail Se concentration ($r = -0.30$, $p = 0.04$). There was no additional decrease in genotoxic stress within the prostate of dogs that had toenail Se concentration in the two highest quartiles (i.e. >0.74 ppm). These findings support the hypothesis that toenails are a readily accessible surrogate tissue to monitor the effects of dietary Se on carcinogenic events within the prostate. The possibility of a threshold for the prostate cancer protective effects of Se that can be assayed non-invasively, warrants further investigation.

Environmental Mutagen Society 33rd Annual Meeting, Anchorage, Alaska, May 2002

Reduction of DNA Damage Within the Prostate of Elderly Dogs Receiving Supranutritional Selenium is Associated with Increased Apoptosis of Epithelial Cells

S. Shen^{1,4}, D.M. Cooley^{1,4}, L.T. Glickman², J.S. Morris³, C. Oteham¹, D. Schlittler¹, D.J. Waters^{1,4}, Departments of Veterinary Clinical Sciences¹ and Veterinary Pathobiology², Purdue University, West Lafayette, IN, 47907; University of Missouri-Columbia Research Reactor Center³, Columbia, MO, 65211; Gerald P. Murphy Cancer Foundation⁴, Seattle, WA 98125;

During the next 12 years, the SELECT trial will study 32,400 men to determine if daily supplementation with selenium ± vitamin E decreases the incidence of prostate cancer. However, the mechanisms by which selenium modulates multistep prostate carcinogenesis remain unclear. Previously, we showed that supranutritional selenium exerts a DNA damage-sparing effect on the aged prostate using the dog model. To test the hypothesis that selenium abrogates the extent of DNA damage within the prostate by upregulating apoptosis of damaged epithelial cells, we studied 30 elderly male beagle dogs randomly assigned to 3 groups: no treatment, 3 μ g/kg selenomethionine daily, and 6 μ g/kg selenomethionine daily. After 7 months, dogs were euthanatized and formalin-fixed prostate tissue sections were labeled with ApopTag™. There was a low level of apoptosis within the prostate of control dogs. In contrast, dogs treated with 6 μ g/kg selenomethionine had a 6.5X increase in the number of apoptotic cells per field compared to control dogs ($p=0.09$). Foci of markedly upregulated apoptosis (hot spots), defined as microscopic fields in which the number of apoptotic cells was at least 30X greater than the average for control dogs, were present in 9 of 20 (45%) selenium supplemented dogs. In contrast, apoptotic hot spots were found in only 1 of 10 control dogs ($p=0.10$). Foci of upregulated apoptosis were present in 4 of 233 (1.7%) and 37 of 463 (8.0%) fields evaluated in control and selenium supplemented dogs, respectively ($p=0.07$). These studies of the dog prostate enable us to explore the anticarcinogenic mechanisms of selenium supplementation in a model of selenium adequacy, which mimics those American men who will participate in the SELECT trial. [Supported by the USAMRMC PCRP Grant PC-970492 awarded to DJW]